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(71) Applicant *(for all designated States except US)*: SMITHKLINE BEECHAM CORPORATION [US/US]; Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: POUPARD, James, Arthur [US/US]; SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).

(74) Agent: WILLIAMS, Jamice, Elaine; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).

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(54) Title: PHARMACEUTICAL FORMULATIONS WITH CLAVULANIC ACID AND AN ANTIMYCOBACTERIAL AGENT

(57) Abstract

Pharmaceutical formulations which comprise a pharmaceutically acceptable salt of clavulanic acid such as potassium clavulanate, in combination with an antimycobacterial agent such as ethambutol, and optionally one or more other antimicrobial agents such as amoxycillin. The formulations are suitable for therapy against infection by mycobacteria.

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PHARMACEUTICAL FORMULATIONS WITH CLAVULANIC ACID AND AN ANTIMYCOBACTERIAL AGENT

This invention relates to pharmaceutical formulations, in particular to novel uses of formulations in connection with the treatment of infection by mycobacteria.

Mycobacteria include M. tuberculosis and nontuberculosis mycobacterial species such as *M. fortuitum, M.kansasii, M.marinum* and *M. avium* complex.

The invention provides a pharmaceutical formulation which comprises a pharmaceutically acceptable salt of clavulanic acid, in combination with an antimycobacterial agent, and optionally one or more other antimicrobial agents.

The formulation of the invention is suitable for use in the treatment of infection by mycobacteria in humans or animals.

The present invention also provides a method of use of a pharmaceutically acceptable salt of clavulanic acid and an antimycobacterial agent, and optionally one or more other antimicrobial agents, together in combination in the manufacture of a medicament formulation, particularly a formulation for the treatment of infection of humans or animals by mycobacteria.

The present invention further provides a method for the preparation of a pharmaceutical formulation as defined above, which method comprises admixing the combination of a pharmaceutically acceptable salt of clavulanic acid, and an antimycobacterial agent and optionally one or more other antimicrobial agents.

As salts of clavulanic acid are extremely hygroscopic such formulations must be prepared in dry conditions, typically at a relative humidity of 30% or less. All constituents of the formulation should be predried. Dry formulations for aqueous reconstitution may be made up with an aqueous vehicle shortly before use.

The present invention further provides a pharmaceutical formulation as defined above for use as an active therapeutic substance, particularly in the treatment of infection of humans or animals by mycobacteria.

The present invention also provides the use of a pharmaceutically acceptable salt of clavulanic acid to enhance the antimycobacterial effectiveness of an antimycobacterial agent.

Further the invention provides a method for the treatment of an infection by mycobacteria in humans or animals, which comprises administering thereto, simultaneously or successively in any order, a pharmaceutically acceptable salt of clavulanic acid and an antimycobacterial agent, and optionally one or more other antimicrobial agents. Typically the clavulanic acid and antimycobacterial agent may be co-administered together, optionally also together with one or more other antimicrobial agents, e.g. as a pharmaceutical composition.

Typical mycobacteria are *M. tuberculosis, M. fortuitum, M. kansasii, M. marinum*, and *M.avium* complex.

The most pharmaceutically stable salt of clavulanic acid is the potassium salt, ie potassium clavulanate.

A suitable antimycobacterial agent is ethambutol [that is, 2.2'-(1,2-ethanediyldiimino)di-1-butanol; d-N,N'-bis(1-hydroxymethylpropyl) ethylene diamine] or derivatives thereof such as the hydrochloride or dihydrochloride.

Suitable optional other antimicrobial agents which may be included in the formulations and methods of this invention as described herein include antibiotics, e.g β-lactam antibiotics such as penicillins and cephalosporins. Suitable antibiotics include those for example listed in GB 1578739, e.g on page 3 line 25 to 36 thereof.

Suitable β-lactam antibiotics include the penicillins: amoxycillin, ampicillin, apalcillin, aspoxicillin, azidocillin, azlocillin, aztreonam, benzylpenicillin, bacampicillin, carbenicillin, cloxacillin, cyclacillin, dicloxicillin, epicillin, flucloxacillin, lenampicillin, mecillinam, methicillin, mezlocillin, phenoxymethylpenicillin, piperacillin, pivampicillin, propicillin, sulbenicillin, talampicillin, and ticarcillin; and the cephalosporins: cefaclor, cefadroxil, cefatrizine, cefclidine, cefamandole, cefazolin, cefbuperazone, cefcanel daloxate, cefdinir, cefepime, cefetamet pivoxil, cefixime, cefminox, cefminoxime,. cefmetazole, cefonicid, cefoperazone, cefotaxime, cefotetan, cefotiam, cefotiam hexetil, cefoxitin, cefpimizole, cefpiramide, cefrirome, cefpodoxime proxetil ,cefprozil, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone, cefuroxime axetil, cefuroxime, cephacetrile, cephalexin, cephaloridine, cephalothin, cephamanadole nafate, cephapirin, cephoperazone, cefsulodin, cefuzonam, cephradine, loracarbef, DQ 2556, ME1207, S-1006, SCE-2787 and moxalactam.

Amoxycillin is a preferred β-lactam antibiotic, for example used in the form of amoxycillin trihydrate or sodium amoxycillin, for example anhydrous crystalline sodium amoxycillin in the form described in EP 0131147.

The clavulanic acid and antimycobacterial agent and other optional antimicrobial agents, as used in this invention, whether in the form of the free acids, salts, esters or derivatives thereof are preferably each in a substantially pure form, e.g. at least 60% pure, more suitably at least 75% pure, preferably at least 85% especially at least 98% pure on a weight basis.

The formulations of the invention may be in a form adapted for oral or parenteral use and may be used for the treatment of infection in humans and animals especially mammals, including in particular domesticated animals (including farm animals).

The formulations of the invention may, for example, be made up in the form of tablets, suspensions, solutions, reconstitutable powders, and sterile forms suitable for injection or infusion. Such formulations may contain conventional pharmaceutically acceptable materials, for example solid or liquid diluents, colours

and preservatives, in accordance with conventional pharmaceutical practice in a manner well understood by those skilled in the art of formulating antibiotics. Normally all such ingredients are predried. Aqueous solutions are normally provided in the form of dry ingredients for reconstitution immediately prior to use.

It can be particularly advantageous for the formulations of the invention to be administered to a patient by injection or infusion. That method of administration has the advantage of rapidly resulting in high blood levels of the active ingredient compounds being administered. Accordingly, in one preferred form of the formulation of the invention, the compounds are present in sterile form, including in sterile crystalline form. A further preferred form of the formulation of the invention, is one in which the formulation is in injectable or infusable form.

One injectable or infusable form of the formulation of the invention is an injectable or infusable solution, which suitably comprises an aqueous solution of a pharmaceutically acceptable salt of clavulanic acid and an antimycobacterial agent, and optionally one or more other antimicrobial agents, in a sterile pyrogen-free liquid, for example water or aqueous ethanol. Because of the water sensitivity of salts of clavulanic acid such a formulation should be provided as the dry constituents and be made up with water immediately prior to use.

A further injectable or infusable form of the formulation of the invention is an injectable or infusable suspension, in which case the salt of clavulanic acid, and antimycobacterial agent are advantageously present in finely particulate form. The suspension may be an aqueous suspension in, for example, sterile water or sterile saline, which may additionally include a suspending agent, for example poly-vinylpyrrolidone. Alternatively, the suspension may be an oily suspension in a pharmaceutically acceptable oil suspending agent, for example arachis oil, which should be dry. Because of the water sensitivity of clavulanic acid salts such aqueous suspensions should be made up from dry constituents immediately prior to use.

A formulation according to the invention may be in unit dosage form, for example unit dosage form for parenteral administration, which will primarily include administration by injection or infusion, especially intramuscular and intravenous administration.

In the formulations and methods according to the invention, the antimycobacterial agent may be administered to the patient in an antibacterially effective amount, and the salt of clavulanic acid may be administered in an amount effective to inhibit β-lactamase enzymes.

The salt of clavulanic acid will generally be administered in an amount sufficient to inhibit the β-lactamase enzyme(s) associated with infecting bacterial and/or mycobacterial organism(s). To that end, it may suitably be administered to the patient at a daily dosage of from 0.3 to 15 mg/kg, preferably from 0.7 to 10 mg/kg,

for example from 0.7 to 7 mg/kg, of body weight. For an adult human (of approximately 70 kg body weight), from 25 to 1000 mg, preferably from 50 to 500 mg, of the salt of clavulanic acid may be administered daily, suitably in from 1 to 6, preferably from 2 to 4, separate doses. Higher or lower dosages may, however, be used in accordance with clinical practice.

When the formulations according to the invention are presented in unit dosage form, each unit dose may suitably comprise from 12.5 to 1000 mg, preferably from 12.5 to 250 mg, of the salt of clavulanic acid. Each unit dose may, for example, be 12.5, 25, 50, 75, 100, 125, 150, 200, or 250 mg of the salt of clavulanic acid.

The ratio of the amount of the salt of clavulanic acid used according to the invention : amount of any other optional antimicrobial agent present may vary within a wide range, e.g 1:1 to 1:30 by weight. In the case of amoxycillin or salts or esters thereof the said ratio may, for example, be from 1:1 to 1:12; more particularly, it may, for example, be from 1:1 to 1:7, 1:1 to 1:4 or 1:1 to 1:2, by weight.

The amount of any optional other antimicrobial agent, e.g amoxycillin or salts or esters thereof in a formulation according to the invention will normally be approximately similar to the amount in which it is conventionally used *per se*. In the case of amoxycillin for example from 125 to 3000 mg per day, and from 125 to 3000 mg per unit dose, advantageously from about 125 to 1000 mg per unit dose, from 2 to 4 times daily may be administered. In the case of ticarcillin for example a maximum of 3.2 g six to eight hourly may be administered.

The amount of antimycobacterial agent in a formulation according to the invention will normally be approximately similar to the amount in which it is conventionally used *per se*. For example in the case of ethambutol up to 25 mg/kg per day may be administered.

An example of a suitable formulation for oral administration according to the invention is one comprising from 12.5 to 250 mg, preferably from 25 to 125 mg, of potassium clavulanate, in admixture or conjunction with 100 to 400 mg of ethambutol or a derivative thereof per unit dose, optionally also comprising 125 to 3000mg of amoxycillin trihydrate.

An example of a suitable formulation for parenteral administration according to this invention is one comprising from 12.5 to 250mg, preferably from 25 to 125 mg, of potassium clavulanate, in admixture or conjunction with 100 to 400 mg of ethambutol or a derivative thereof per unit dose, optionally also comprising 125 to 3000 mg of sodium amoxycillin.

The following example illustrates the synergistic antibacterial activity of a combination of a salt of clavulanic acid and the antimycobacterial agent ethambutol, together with the antimicrobial agent amoxycillin, and compares this combination with the activity of amoxycillin and a salt of clavulanic acid alone, and of thambutol

alone, against mycobacteria.

Example 1.

The susceptibility of four mycobacterial species to amoxycillin plus clavulanate, and ethambutol was determined. Microdilution MIC methodology was used to test 24 *M. fortuitum, M. kansasii* and *M. marinum* strains and BACTEC methodology was used to test 20 *M. avium* complex (MAC) isolates.

Five of the nine *M. fortuitum*, four of the ten *M. kansasii* and three of the five *M. marinum* isolates had amoxycillin/clavulanate MIC's of ≤ 8/4 mcg/ml. Ten of the twenty MAC isolates had amoxycillin/clavulanate MIC results ≤ 8/4 mcg/ml, with eight of the isolates having MIC's of ≤ 4/2 mcg/ml. Amoxycillin alone and clavulanate alone were relatively ineffective against these isolates. When amoxycillin/clavulanate was combined with 2 mcg/ml of ethambutol, the susceptibility of seven *M. kansasii* and four *M. marinum* isolates was increased compared to either amoxycillin/clavulanate or ethambutol alone. The data obtained indicate that the concentrations of amoxycillin/clavulanate alone and in combination with ethambutol required to inhibit mycobacterial growth *in vitro* are within the therapeutic range of amoxycillin/clavulanate.

The data presented below in Tables 1 and 2 shows MIC (μg/ml) results for *M. kansasii* and *M. marinum*. MICs were determined in middlebrook broth with OADC (oleic acid, albumin, dextrose and catalase) enrichment inocculated with 5 x 10^4 CFU/ml of the mycobacteria. After sufficient growth the MICs were determined as the lowest concentration of antibiotic(s) which inhibited growth.

In Tables 1 and 2, clav. = clavulanate alone, amx. = amoxycillin alone, etham. = ethambutol alone, clav/amx = clavulanate + amoxycillin, clav/amx/etham = clavulanate + amoxycillin + ethambutol.

Table 1: Myc bacterium Kansasii.

Isolate	clav.	amx.	clav/amx	etham.	clav/amx /etham.	clav/amx /etham.
258A	64	>256	8/4	16	<0.25/ 0.125/1	<0.25/ 0.125/2
475A	64	>256	8/4	16	<0.25/ 0.125/1	<0.25/ 0.125/2
506A	>128	>256	64/32	16	16/8/1	8/4/2
626A	32	>256	16/8	16	<0.25/ 0.125/1	<0.25/ 0.125/2
640A	32	256	8/4	16	<0.25/0.1 25/1	<0.25/ 0.125/2
1220A	32	64	8/4	8	<0.25/ 0.125/1	<0.25/ 0.125/2
1609A	128	>256	32/16	8	<0.25/ 0.125/1	<0.24/ 0.125/2
1610A	32	>256	32/16	16	4/2/1	2/1/2
1722A	64	>256	16/8	8	2/1/1	2/1/2
1870A	64	>256	16/8	16	<0.25/ 0.125/1	<0.25/ 0.125/2

Table 2: Mycobacterium Marinum.

Isolate	clav.	amx.	clav/amx	etham.	clav/amx /etham	clav/amx /etham
1508A	64	256	16/8	8	<0.25/	<0.25/
2084A	32	>256	8/4	4	0.125/1 <0.25/	0.125/2 <0.25/
2162A	32	>256	8/4	4	0.125/1 <0.25/	0.125/2 <0.25/
4935A	32	128	8/4	4	0.125/1 <0.25/	0.125/2 <0.25/
4939A	32	>256	16/8	8	0.125/1 2/1/1	0.125/2 1/0.5/2

Claims:

1. A pharmaceutical formulation comprising a pharmaceutically acceptable salt of clavulanic acid, in combination with an antimycobacterial agent, and optionally with one or more other antimicrobial agents.

2. A formulation according to claim 1 wherein the pharmaceutically acceptable salt of clavulanic acid is potassium clavulanate.

3. A formulation according to claim 1 wherein the antimycobacterial agent is ethambutol.

4. A formulation according to claim 1 wherein an additional antimicrobial agent is present which is a β-lactam antibiotic.

5. A formulation according to claim 4 wherein the β-lactam antibiotic is amoxycillin, present as the free acid, or as a pharmaceuticaly acceptable salt or ester thereof.

6. A formulation according to claim 4 wherein the ratio pharmaceutically acceptable salt of clavulanic acid : β-lactam antibiotic is in the range 1:1 to 1:30.

7. A formulation according to claim 1 in unit dosage form comprising 12.5 to 1000 mg of a pharmaceutically acceptable salt of clavulanic and 100 to 400 mg of ethambutol or a derivative thereof.

8. A formulation according to claim 7 additionally comprising 125 to 3000 mg of amoxycillin or a derivative thereof.

9. A formulation according to claim 1 for parenteral administration.

10. A formulation according to claim 1 for oral administration.

11. A formulation according to claim 1 for use in the treatment of infection by mycobacteria in humans or animals.

12. A method of use of a pharmaceutically acceptable salt of clavulanic acid, and an antimycobacterial agent, and optionally one or more other antimicrobial agents,

together in combination in the manufacture of a medicament formulation.

13. A method according to claim 11 comprising admixing the combination pharmaceutically acceptable salt of clavulanic acid, an antimycobacterial agent, and optionally one or more other antimicrobial agents.

14. A pharmaceutical formulation according to claim 1 for use as an active therapeutic substance.

15. The use of a pharmaceutically acceptable salt of clavulanic acid to enhance the antimycobacterial activity of an antimycobacterial agent.

16. A method for the treatment of an infection by mycobacteria in humans or animals, which comprises administering thereto, simultaneously or successively in any order, a pharmaceutically acceptable salt of clavulanic acid and an antimycobacterial agent and optionally one or more other antimicrobial agents.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/42 A61K31/13 //(A61K31/42,31:13)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INDIAN J. MED. RES. vol. 88 , August 1988 pages 118 - 123 C. BHATTACHARYA ET AL. 'Comparison of sensitivity of Mycobacterium spp. to combinations of clavulanic acid & penicillins with certain antitubercular agents' see table II ---	1-16
Y	DRUGS EXP. CLIN. RES. vol. 14, no. 12 , 1988 pages 741 - 745 M. CASAL ET AL. 'In vitro activity of antimicrobial agents aginst mycobacteria' see the whole document ---	1-16

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[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
31 August 1994	13.09.94

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AM. REV. RESPIR. DIS. vol. 145, no. 3 , 1992 pages 657 - 660 Y. ZHANG ET AL. 'Beta-lactamase inhibitors and the inducibility of the beta-lactamase of Mycobacterium tuberculosis' see the whole document ---	1-16
Y	CHEST vol. 99, no. 4 , 1991 pages 1025 - 1026 J. NADLER ET AL. 'Amoxicillin-Clavulanic acid for treating drug-resistant Mycobacterium tuberculosis' see the whole document -----	1-16